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Abstract

Seagrass meadows provide important ecosystem services and are critical to the survival of the associated invertebrate community. However, they are threatened worldwide by human-driven environmental change. Understanding the seagrasses' potential for adaptation is critical to assess not only their ability to persist under future global change scenarios, but also to assess the persistence of the associated communities.

Here, we screened wild population of *Posidonia oceanica*, an endemic long-lived seagrass in the Mediterranean Sea, for genes that may be target of environmental selection, using an outlier and a genome-wide transcriptome analysis. We identified loci, which polymorphism or differential expression was associated with either a latitudinal or a bathymetric gradient, as well as with both gradients, in an effort to identify loci associated with temperature and light. We found candidate genes underlying growth and immunity to be divergent between populations adapted to different latitudes and/or depths, providing evidence for local adaptation. Furthermore, we found evidence of reduced gene flow among populations, including adjacent populations. Reduced gene flow, combined with low sexual recombination, small effective population size, and long generation time of *P. oceanica*, raises concerns for the long-term persistence of this species, especially in the face of rapid environmental change driven by human activities.

52 **Keywords**

53 outliers, transcriptome, candidate genes, adaptation, priority species,

54 Mediterranean Sea

55

Introduction

Organisms have historically responded to changes in the environment by migrating, tolerating or adapting (Hessen *et al*, 2013). However, human activities exacerbate environmental changes, imposing shifts in ecological niches that often surpass the adaptive potential of species (Hoegh-Guldberg *et al*, 2007) and lead to local extinctions (Cardinale *et al*, 2012; Smith *et al*, 2007). Stress imposed by temperature changes has been associated with loss of biodiversity (Both *et al*, 2006; Corlett and Westcott, 2013; Franks *et al*, 2014; Van Der Wal *et al*, 2013), with severe consequences on sessile species (Rivetti *et al*, 2014). Indeed, range reduction of the large majority of benthic marine species has been associated with global warming (Bay and Palumbi, 2014; Jueterbock *et al*, 2016; Sanford and Kelly, 2011). However, because of the complex interplay among temperature and other environmental stressors, it remains a challenge to disentangle the impact of temperature stress from the one of other environmental factors.

Here, we investigated local adaptation of populations of the seagrass *Posidonia oceanica* sampled along a latitudinal and a bathymetric gradient, using a genome scan and a transcriptome analysis. Temperature and light are key environmental factors varying along these gradients; in particular light is strongly associated with the bathymetric gradient. We hypothesized that a signature of local adaptation shared between the bathymetric and the latitudinal gradient reflects adaptation to temperature, while a signature of

local adaptation associated with the bathymetric gradient reflects adaptation to light cues. To test these hypotheses, we looked mined for outlier loci uniquely associated with the bathymetric or latitudinal gradient, as well as for shared outlier loci between gradients. Furthermore, we performed a genome-wide differential expression analysis to identify candidate genes and gene pathways associated with the bathymetric gradient.

Seagrasses are one among the most valuable ecosystems on earth, as they are important providers of ecosystem services and sustain many invertebrate and vertebrate communities (Costanza *et al*, 1997; Giakoumi *et al*, 2015). Understanding how seagrasses respond to environmental change is critical not only to assess their ability to persist to future global change, but also to assess the persistence of the associated communities. The seagrass *Posidonia oceanica* (L.) Delile is an endemic long-lived seagrass in the Mediterranean Sea. Individual shoots of this plant survive for decades (Short *et al*, 2011), and grow via horizontal rhizome elongation (1 to 10 cm per year; Marbà and Duarte, 1998). Single genotypes can persist for millennia via asexual reproduction (Arnaud-Haond *et al*, 2012; Ruggiero *et al*, 2002). Flowering via sexual reproduction is extremely heterogeneous at spatial and temporal scales (Diaz-Almela *et al*, 2006; Jahnke *et al*, 2015a). Moreover, the establishment success of flowers is low (Balestri and Cinelli, 2003; Diaz-Almela *et al*, 2006). *P. oceanica* populations in the Mediterranean Sea show pronounced genetic structure (Arnaud-Haond *et al*,

2007; Serra *et al*, 2010), with reduced gene flow both across latitudes (Procaccini *et al*, 2002) and depths (Migliaccio *et al*, 2005; Procaccini *et al*, 2001). Low genetic diversity has been observed at regional and global scale (Arnaud-Haond *et al*, 2007; Serra *et al*, 2010). Furthermore, low genetic and genotypic diversity have been associated with local or regional extinctions (Jahnke *et al*, 2015b). These studies support predictions of *P. oceanica* functional extinction from the Mediterranean Sea under projected global warming (Jorda *et al*, 2012).

Materials & Methods

Sampling design

We collected *P. oceanica* populations from six localities in the Mediterranean Sea along a latitudinal (1,000 Km, Fig. 1) and a bathymetric gradient. Each locality was sampled at two depths (5 m and 20 – 25 m) (Table S1). Average sea surface temperature (SST) records from the last three decades were provided by Copernicus (SST_MED_SST_L4_REP_OBSERVATIONS_010_021_a_1438858783944) (Nardelli *et al*, 2013). Using these records, we calculated average temperature differences among populations (Table S2). Historical records show differences of up to 10°C between the two sampled depths, in particular when vertical mixing is prevented by the presence of the summer thermocline (Marin-Guirao *et al*, 2016). This temperature difference is less

pronounced in winter. For one of the sampled locations (Stareso, Table S1), the photosynthetic active radiation (PAR) was recorded at the time of sampling (1pm), and corresponded to 430 and 92 μmol m^{-2} s^{-1} at 5 and 20 m, respectively (Procaccini *et al*, 2017). Comparable differences in irradiance between depths can be expected at the other sampled locations. However, PAR data are not available for all locations.

At each site and from each depth, between 20 and 31 adult leaves of *P. oceanica* were sampled non-destructively (Table S1). The mean distance between samples at each site ranged between 5 and 8 m to reduce the chance of collecting identical clones (Arnaud-Haond *et al*, 2007; Serra *et al*, 2010). After collection, the leaves were cleaned from epiphytes and stored in silica gel, prior to DNA extraction. All samples were genotyped at 23 putatively neutral microsatellite loci, 14 of which were EST-linked (Table S3; Alberto *et al*, 2003; Arranz *et al*, 2013; Migliaccio *et al*, 2005, Procaccini and Waycott, 1998).

The Stareso population (Corse, 8°45'E,42°35'N, Fig. 1) was previously used to generate a reference transcriptome for *P. oceanica* (D'Esposito *et al*, 2016). Here, we used the available RNA-Seq data to identify differentially expressed genes between the shallow and deep stands of the same population. As samples were collected at two time points within the same day (12 noon and 6.30 pm), we also studied plastic response in genome-

wide expression in response to light by identifying differential gene expression between the samples collected at the same depth at different time points. At the time of sampling, sunrise was at 7.25 am and sunset was at 6.45 pm.

Population response to environmental change – outlier analysis

DNA was extracted from 271 samples (*ca.* 20 mg of dried tissue per sample) collected from the six localities at two depths (Table S1) using the NucleoSpin® 96 Plant II kit (Macherey-Nagel), following a modified protocol optimized for a Biomek FX robotic station (Tomasello *et al*, 2009). Genotyping was performed using an ABI Prism 3730 automated DNA sequencer (Applied Biosystems), following PCR amplification, with the following cycling: 95ºC for 15min, 35 cycles of 94ºC for 30 sec, 60ºC for 1 min 30 sec and 72ºC for 1 min, with a final extension step of 60 ºC for 30 min. Only samples that were successfully genotyped at ≥ 90% of the loci (22/24 loci) were used for downstream analyses.

Although the likelihood of sampling the same genotype was low due to our sampling design, we screened for the presence of identical multilocus genotypes (MLGs) using the psex (F_{IS}) (the probability that the repeated genotypes originate from distinct sexual reproductive events considering departures from Hardy–Weinberg equilibrium) in GenClone (Arnaud-

164 Haond and Belkhir, 2007). When identical MLGs were detected, only one
165 was retained within each site for downstream analyses. Our data-set
166 comprised 237 individuals (18-22 individuals per population, Table S1).

167 All genotyped populations were used in an outlier analysis to
168 identify loci putatively associated with the latitudinal and depth gradients.
169 We contrasted pairwise populations at the extreme of the latitudinal
170 gradient. In addition, we contrasted stands of the same population sampled
171 at 5m and 25m. Prior to this analysis, we performed a population genetic
172 structure analysis, following Orsini *et al.* (2012). Specifically, we performed
173 an analysis of population genetic differentiation in Arlequin3.5 with 10,000
174 permutations (Excoffier and Lischer, 2010) and a population genetic
175 structure analysis using a Discriminant Analysis of Principal Component
176 (DAPC) (Jombart *et al*, 2010) to identify the number of independent
177 populations present in the dataset. We performed the DAPC implemented in
178 Adegenet (Jombart, 2008) in R 3.2.2. (R Development Core Team, 2012)
179 using eight principal components as suggested by the a-score optimization
180 analysis. The analysis of population differentiation and the genetic
181 discrimination identified a northern and a southern population cluster, and
182 identified as independent populations stands of the same populations
183 sampled at different depths (Fig. 2). This separation is also evident from the
184 genetic differentiation analysis (Table S4). Hence, for the downstream
185 analysis we considered populations from the same locality at different

depths as independent, and grouped populations along the latitudinal transect into a northern and a southern group.

To identify loci putatively linked to environmental factors, we used multiple pairwise population comparisons. This approach has been previously shown to reduce the number of false positives, by showing parallel patterns of locus-specific variation among replicate population comparisons (Orsini *et al*, 2012). To identify loci associated with the depth gradient, we contrasted stands at different depths of the same population, and a pool of all shallow vs all deep stands (Table S5). To identify loci associated with the latitudinal gradient, we performed pairwise population comparisons separately for the shallow and deep stands. For this analysis, we contrasted three random population pairs from the northern and the southern groups, as well as the pool of northern versus southern populations at the two depths (Table S6). With this approach, we were able to identify putative outlier loci shared between the bathymetric and latitudinal gradients as well as outlier loci unique to either gradient. The outlier analysis was conducted with Lositan (Antao *et al*, 2008) and BayeScan (Foll and Gaggiotti, 2008). To obtain Lositan results we run simulations for 50,000 iterations, and used a 95% confidence interval. We performed the BayeScan analysis with default settings and used the provided R script to identify loci showing significant deviation from expectations under neutrality by plotting their posterior distribution. Using a conservative approach to reduce false

positives (Orsini *et al*, 2012), we plotted the number of times loci were detected as outliers in the pairwise population comparisons. To decide on the minimum threshold needed to define an outlier as a locus potentially under selection, we plotted the frequency with which every locus was detected as an outlier in pairwise analyses of population comparisons. Loci falling outside the 95% boundaries of this frequency distribution were considered 'real' outliers. Loci present only in comparisons from the bathymetric or latitudinal gradient are hereafter referred to as "bathymetric loci" and "latitude loci", respectively. Loci common to the two gradients are referred to as "gradient-shared loci".

Association of neutral and non-neutral genetic variation with the environment

The outlier analysis allowed us to distinguish neutral from putatively non-neutral loci. These two categories of loci were used in a correlative analysis with temperature and geographic distance. For this correlative analysis, we used the following categories of loci: 1) strictly neutral (not identified as outliers in any of the gradients); 2) gradient-shared; 3) latitude loci; and 4) bathymetric loci. On these four categories of loci we measured the partitioning of molecular variance via AMOVA with GenAlEx 6.5 (Peakall and Smouse, 2012) using 1,000 permutations. Three hierarchical levels were

used: a) depth (populations at two depths) or latitude (North and South populations, separately for shallow and deep stands); b) populations within groups, where the groups were either populations at the same depth or at the same latitude and; c) individuals within populations.

The allele frequencies of loci calculated by shallow and deeps stands, as well as by northern and southern populations are listed in Table S7.

We used ArcGIS 10.1® (ESRI) to extract the sea surface temperature (SST) values for our sampling locations and measured geographic distances between sampling locations using the shortest path over the sea without crossing land. We performed a Multiple Matrix Regression with Randomization analysis (MMRR) (Wang, 2013) using R to identify correlations between genetic distance (measured as F_{ST} at both neutral and outlier loci), geographic distance, and temperature (measured as the difference between locations of averaged temperature between 1981 and 2012, the period for which SST is available). MMRR uses a randomized permutation procedure to correct for possible dependency between geographic distance and environmental variables. As compared to a classic partial Mantel test, this method reduces type I errors (Wang, 2013). We used 10,000 permutations to test for significant correlations among the standardized matrices of genetic distance, geographic distance and temperature for shallow populations only, as only superficial SST temperatures were available.

Functional annotation of outlier loci

The sequences of the outlier loci associated with the gradients were used for functional annotations after masking for low complexity regions, which improves gene homology searches. The annotation was done by blasting the sequence of the outlier loci in the NCBI databank and against the reference transcriptome of *P. oceanica* (D'Esposito *et al*, 2016) using blastx (Altschul *et al*, 1997) followed by function query in the EMBL-EBI Pfam data base (Bateman *et al*, 2004).

Population response to environmental change – evolutionary and plastic differences in gene expression

We used RNA-Seq data previously generated to assemble the *P. oceanica* reference transcriptome (D'Esposito *et al.*, 2016). Transcriptome data were available from the Stareso population. Specifically, two biological replicates, collected at two different times of the day (12 noon and 6.30 pm) at two depths (5 m and 20 m depth) were available. PAR at 12 noon was 350 μmol m^{-2} s^{-1} at -5 m, and 100 μmol m^{-2} s^{-1} at -20 m. PAR values at 6.30 pm were 30 μmol m^{-2} s^{-1} at -5 m and zero at -20 m, respectively (Procaccini *et al*, 2017).

We mapped the transcripts onto the reference transcriptome of *P. oceanica* using Bowtie (Langmead *et al*, 2009) and performed a differential

expression analysis between shallow and deep stands, as well as between sampling times at the same depth (Padj = 0.01) with DESeq2 (Love *et al*, 2014). We calculated the Biological Coefficent of Variation (BCV) on the overall samples using edgeR (McCarthy *et al*, 2012). For this analysis, only genes with read counts > 1M in at least two samples were used. The clustering of samples was visualized in an MDS plot using the plotMDS function in edgeR. Genome-wide differential expression profiles were visualized with heatmaps plotted using heatmap.2 from the R package '*gplots*'. Raw zeta-scores were calculated from the DESeq2 normalized count using the R package '*recommenderlab*'.

Results

Population response to environmental change – outlier analysis

The outlier analysis identified two "gradient-shared loci" (Pooc-PC047G07 and Pooc-50), four "bathymetric loci" (Pooc-PC047G07, Pooc-50, Poc-45, and Pooc-PC003H09, Table S5) and three "latitude loci" (Pooc-PC047G07, Pooc-50 and Pooc-PC045G11, Table S6).

Gradient-shared loci showed similarity to genes with known function. The locus Pooc-50 showed significant similarity to the pEARLI1-like protein 3 (accession Q9SU33.1), a protein associated with membrane lipid transfer in *Zostera marina* (accession KMZ61949). The locus Pooc-

293 047G07 showed significant similarity to a 50S ribosomal protein L35
294 (accession PF01632 for structural domain, P23326.1 for gene annotation),
295 one of the large subunits of the ribosome. The frequency of the major allele
296 at the outlier-shared loci was the same in both gradients (Table S7).
297 The outlier Pooc-PCo45G11, associated with latitude, showed significant
298 similarity to the highly conserved eukaryotic translation initiation factor 5A-
299 1 (accession: P69039.1), which is also a conserved domain (PTZ00328).
300 The bathymetric locus Poc-45 did not show similarity to a gene with known
301 function, whereas the bathymetric locus Pooc-PC003H09 showed high
302 similarity to PR-1, a plant protein associated with pathogens defence
303 mechanisms in plants (accession: P33154.1).
304

305 *Association of neutral and non-neutral genetic variation with the*
306 *environment*

307 The analysis of molecular variance (AMOVA) was performed on four
308 categories of loci: 1) strictly neutral (not identified as outliers in any of the
309 gradients); 2) gradient-shared; 3) latitude loci; and 4) bathymetric loci.
310 Overall, the molecular variance showed different patterns for neutral and
311 outlier loci (Table 1). The proportion of molecular variance for all four sets
312 of loci was highest at within population level (Table 1). The molecular
313 variance at within group level was significant for neutral loci and
314 comparable between the depth (22%) and the latitudinal gradients (17% and

315 19% -the two depths are analysed separately, Table 1). For these loci, the
316 among-groups variance was small yet significant (Table 1). The variance of
317 loci associated with latitude was highest at within-population level; for this
318 gradient, 4% of the variance of outlier loci was associated with group
319 variation (Table 1). All molecular variance in the bathymetric outliers was
320 explained at within-population level (Table 1).

321 The analysis of correlation between genetic distance, temperature
322 and geographic distance conducted on the shallow populations (MMRR)
323 showed that temperature was significantly correlated with neutral genetic
324 variation, but not with genetic variation at the outlier loci (Table 2). This
325 analysis also showed that geographic distance did not correlate with genetic
326 distance among populations neither at neutral nor at outlier loci. Geographic
327 distance correlated singificantly with sea surface temperature (p-value =
328 0.03).
329

330 *Population response to environmental change – evolutionary and plastic*
331 *differences in gene expression*

332 The genome-wide differential expression analysis was conducted on stands
333 of the Stareso population. This analysis identified a total of 2,059
334 differentially expressed genes between stands, of which 1,565 were up-
335 regulated and 494 were down-regulated (Table S8). Variability in gene
336 expression was high (BVC = 38%) among samples. The MDS plot

following this analysis revealed a clear separation between shallow and deep stands; moreover, it clustered samples from the same depth (Fig. S1).

A large proportion of the differentially expressed genes identified by the DESeq2 analysis belonged to five functional categories: transcription factors, metabolic genes, cell wall remodeling, and signaling pathways (Fig. 3, Table S8). The remainder of the differentially expressed genes not falling in these functional categories is listed in Table S8 and includes a number of uncharacterized proteins. Generally, the two deep samples (taken at the same location but at two different times: 12 noon and 6.30pm) showed higher similarity than the two shallow samples in the number of expressed genes and the direction of change in expression (Fig. 3). Differences in genome-wide gene expression between shallow and deep stands were mainly associated with constitutive differential expression of bLHL (basic-helix-loop-helix), cell wall genes and MYB (Fig. 3 and Table S8).

A total of 121 transcription factors (TF) were identified in our analysis, falling into four main functional domains, WRKY, MYB, bHLH, and Ethylene-responsive genes. MYB and bHLH were largely upregulated in the shallow stand, whereas WRKY transcription factors were largely upregulated in the deep stand (Fig. 3). The transcription factors identified in our analysis have diverse biological functions ranging from disease resistance, abiotic and biotic stress response, senescence, development,

differentiation, and metabolism (Table S6). In particular, bHLH is a DNA binding protein involved in flavonoid biosynthesis (Hichri et al., 2011).

Phenylpropanoids, flavonoid biosynthesis and lignin biosynthesis coding genes were highly represented in the differential expression analysis (Fig. 3, Table S8). In the phenylpropanoid pathway we identified genes coding for cinnamic acid 4-hydroxylase (C4H) and 4-coumarate-coenzyme-A-ligase enzyme (4CL) (Table S8). In the flavonoid biosynthesis pathways, we identified genes coding for chalcone isomerase (CHI) and chalcone synthase (CHS), dihydroflavonol-4-reductase, flavanone-3-hydroxylase, leucoanthocyanidin reductase and anthocyanidin reductase. In the lignin biosynthesis pathway we identified innamic-acid-4-hydroxylase, 4-coumarate-CoA-ligase, Shikimate-O-hydroxycinnamoyltransferase, caffeoylshikimate esterase, caffeic acid 3-O-methyltransferase and shikimate O-hydroxycinnamoyltransferase (Table S8).

Most of the transcripts involved in secondary metabolism and cell wall remodeling were upregulated in the shallow population (Fig. 3, Table S8). Among the genes involved in cell wall remodeling we identified genes coding for xyloglucan endotransglucosylase (XET), expansins and cellulose synthase. Finally, genes involved in phosphorylation or de-phosphorylation were the fourth most abundant category retrieved in our analysis. A large proportion of phosphatases was upregulated in the shallow population (Fig. 3).

Discussion

We investigated natural populations of *P. oceanica* with the aim of identifying signature of natural selection in response to key environmental factors varying along latitude and depth, such as light and temperature. Our hypothesis testing was that loci shared between the bathymetric and latitudinal gradient were candidates for adaptive responses to temperature, a factor common to the two gradients. Conversely, loci associated with the bathymetric gradient were likely associated with adaptation to different light regimes.

The candidate loci identified in the outlier analysis, and shared between the bathymetric and latitudinal gradient, include a ribosomal protein (Pooc-047G07, 50S ribosomal protein L35) and a lipid transfer protein 3 (Pooc-50, pEARLI1-like lipid transfer protein 3), whose functions are potentially associated with both temperature and photoperiod. L35 is a structural constituent of the ribosomes, and is among the cellular proteins differentially regulated under stressful conditions (see review Kosovà *et al*, 2018). In bacteria, L35 is commonly downregulated under thermal stress (e.g. Nevarez *et al*, 2008). Although mechanisms of stress response differ among taxa, L35 may be potentially involved in stress response in *P. oceanica*. The protein pEARLI1-like lipid transfer protein 3 is a member of the PRP (proline-rich protein) family, which has multiple functions in plants: i) regulation of flowering time and lignin synthesis (Shi *et al.* 2010);

ii) protection of the plasma membrane and cell wall against low temperature (Zhang and Schläppi 2007); iii) and resistance to fungal infection. Relevant to our findings is that EARLI1 can be activated by temperature and changes in photoperiod (Burier and Schläppi 2004).

Other candidate loci associated with the bathymetric and latitudinal gradients include the eukaryotic translation initiation factor 5A-1 (Pooc-PCo45G11) and a plant pathogen protein (Pooc-PC003H09). Translation initiation factors have been shown to play a fundamental role in growth and development of plants by regulating cell division, cell growth, and cell death (Feng *et al*, 2007; Hopkins *et al*, 2008). Plant pathogenesis-related proteins, such as the PR-1 identified here, are highly expressed in plants after infections and act as an anti-fungal agent (Van Loon *et al*, 2006). Interestingly, the translation initiation factor 5A-1 is involved in regulation of cellular processes underlying both plant development and programmed cell death (Hopkins *et al*, 2008), and in response to heat stress (Xu *et al.* 2011).

The number of loci used in the outlier analysis was small, hence we had limited power in identifying shared loci between gradients. A genome-wide polymorphism analysis will likely alleviate the limitations of the current study. Overall, the candidate genes identified in the current study regulate central metabolism and cell functions, such as growth and development, or are associated with pathogens response. Hence, they do not

have a direct link to light or temperature. However, since the link between temperature and metabolism in the context of climate change is well-recognized (Tewksbury *et al*, 2008), the candidate genes identified here may be indirectly linked to temperature. For example, cell wall hardening has been suggested to alleviate heat stress in in *Posidonia oceanica* (Marín-Guirao *et al*, 2017) as well as in other seagrass species (e.g. *Zostera marina*) (Franssen *et al*, 2014; Jueterbock *et al*, 2016). Follow up experiments under controlled laboratory conditions are required to establish a causal association between the putative candidate genes identified here and temperature.

We observed significant divergence in genome-wide gene expression between shallow and deep stands of the Stareso population. Furthermore, we observed a significantly larger divergence between the two shallow than between the two deep samples. Divergence in genome-wide gene expression between shallow and deep stands is likely explained by evolutionary differences in basal gene expression, whereas differences in gene expression between samples collected at the same depth suggests plastic responses to changing irradiance and temperature. Irradiance measured at the Stareso site was markedly different between the two sampling times. Recent studies support the notion that *P. oceanica* living along the bathymetric gradient have differential resilience and are locally adapted to their local environment (Dattolo *et al*, 2017; Marin-Guirao *et al*,

446 2017). Shallow populations of *P. oceanica* show faster and higher induction
447 of heat-response gene, a potential form of local adaptation to higher
448 temperature regimes (Marin-Guirao *et al*, 2016).

449 Differentially expressed genes between depths were enriched for
450 flavonoid biosynthesis and lignin biosynthesis coding genes. These are
451 metabolism-linked genes responsible for a wide range of biochemical
452 pathways providing plants with secondary metabolites (Weisshaar and
453 Jenkins, 1998). Also enriched were genes involved in cell wall loosening.
454 These latter genes were generally upregulated in the shallow stand, and
455 downregulated in the deep stand, suggesting that bathymetric pressure
456 affects the populations of *P. oceanica* studied here. Finally, differentially
457 regulated genes between depths included lignin coding genes, the second
458 most abundant component of plant cell-walls, as well as xyloglucan
459 endotransglucosylase (XET), expansins and cellulose synthase, These genes
460 are commonly associated with cell wall remodeling.

461 A clear genetic structure was observed both among stands of the
462 same population and among populations. In addition to clear population
463 genetic structure, we observed significant population differentiation (F_{ST}),
464 confirming reduced gene flow among populations. The population genetic
465 structure was not explained by geographic distance as neither neutral nor
466 non-neutral genetic variation were significantly correlated with geographic
467 distance (no Isolation by Distance, IBD). These findings were in line with

previous studies (Arnaud-Haond *et al*, 2007; Dattolo *et al.* 2017; Serra *et al*, 2010). Conversely, neutral genetic variation was significantly correlated with temperature. The patterns of correlation observed here can be explained by a scenario of Isolation by colonization (IBC,Orsini *et al*, 2013), determined by the genetic pattern of early colonization and fuelled by low establishment success of immigrants. This scenario is supported by observations of limited gene-flow among meadows (Arnaud-Haond *et al*, 2007; Jahnke *et al*, 2017; Serra *et al*, 2010) and high levels of clonal reproduction in *P. oceanica* (Arnaud-Haond *et al*, 2012; Serra *et al*, 2010). An IBC scenario agrees with the concept of the "founder takes all" put forward by Water *et al.* (2013). The "founders takes all" scenario suggests that density-dependent processes are likely important in constraining dispersal in the marine environment and play a key role in determining local genetic structure, even in highly connected ecosystems that would otherwise promote panmixia (Waters *et al*, 2013).

Alternatively, the observed population genetic differentiation observed in our study can be explained by endogenous selection on intrinsic genetic incompatibilities (Bierne et al 2011). High genetic structure and low gene flow among populations of *P. oceanica* may be indicative of hybrid fitness depression or endogenous selection again migrants (Bierne 2002). Laboratory crosses and fitness analysis across multiple generations is required to validate this hypothesis.

Conclusions

The candidate genes associated with depth and/or latitude underpin central metabolic, cell remodelling and immunoregulation functions. Given the demonstrated link between metabolism and temperature, most of the assessed genes may be indirectly associated with temperature response. Moreover, some of the candidate genes associated with both gradients have been previously linked to temperature and/or light.

The analysis of genome-wide differential gene expression clearly identified divergence in basal expression of five protein functional categories. This divergence is likely explained by adaptation to the local environment. Evidence for reduced gene flow among populations and among stands of the same population at different depths, combined with low sexual recombination, small effective population size, and long generation time of *P. oceanica*, pose concerns for the long-term persistence of this species, especially in the face of rapid environmental change driven by human activities. These concerns are supported by a recent meta-analysis suggesting that *P. oceanica* meadows with low genetic and genotypic diversity are unable to persist in highly impacted areas (Jahnke *et al*, 2015b).

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Conflict of Interest: The authors declare that they have no competing interests

Data Archiving: Microsatellites genotypes are deposited at dryad entry XXX. The reference transcriptome of *P. oceanica* is available at NCBI (Accession number:GEMD01000000). The data generated in this amanuscript are available at the NCBI Sequence Read Archive (SRA) under the accession number SRR3289754, SRR3289740, SRR3289755 and SRR3289704

Authors' contributions: MJ performed the population genetic analyses and wrote the first manuscript draft. DDE and ED performed the genotyping. DDE also performed RNA extraction for the transcriptomic analysis. LO (Luigi Orru') and AL performed the DE analysis and the functional gene annotation. GP and FB conceived the sampling strategy along the latitudinal gradient. FB coordinated and performed the sampling of two populations.

SM and GP conceived the transcriptomic work. GP conceived the study and coordinated the experimental work. LO (Luisa Orsini) coordinated data analysis and manuscript writing. All authors contributed to manuscript editing.

Supplementary information is available on *Heredity*'s website

References

Alberto F, Correia L, Arnaud-Haond S, Billot C, Duarte CM, Serrão EA (2003). New microsatellite markers for the endemic Mediterranean seagrass *Posidonia oceanica*. *Molecular Ecology Notes* **3**(2)**:** 253-255.

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W *et al* (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**(17)**:** 3389-3402.

Antao T, Lopes A, Lopes R, Beja-Pereira A, Luikart G (2008). LOSITAN: a workbench to detect molecular adaptation based on a Fst-outlier method. *BMC bioinformatics* **9**(1)**:** 323.

Arnaud-Haond S, Belkhir K (2007). genclone: a computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. *Molecular Ecology Notes* **7**(1)**:** 15-17.

Arnaud-Haond S, Duarte CM, Diaz-Almela E, Marbà N, Sintes T, Serrão EA (2012). Implications of extreme life span in clonal organisms: millenary clones in meadows of the threatened seagrass *Posidonia oceanica*. *PloS one* **7**(2)**:** e30454.

Arnaud-Haond S, Migliaccio M, Diaz-Almela E, Teixeira S, van de Vliet MS, Alberto F *et al* (2007). Vicariance patterns in the Mediterranean Sea: east-west cleavage and low dispersal in the endemic seagrass *Posidonia oceanica*. *Journal of biogeography* **34**(6)**:** 963-976.

Arranz SE, Avarre J-C, Balasundaram C, Bouza C, Calcaterra NB, Cezilly F *et al* (2013). Permanent Genetic Resources added to Molecular Ecology

Resources Database 1 December 2012–31 January 2013. *Molecular Ecology Resources* **13**(3)**:** 546-549.

Balestri E, Cinelli F (2003). Sexual reproductive success in *Posidonia oceanica*. *Aquatic Botany* **75**(1)**:** 21-32.

Bateman A, Coin L, Durbin R, Finn RD, Hollich V, Griffiths-Jones S *et al* (2004). The Pfam protein families database. *Nucleic Acids Research* **32**(suppl 1)**:** D138-D141.

Bay RA, Palumbi SR (2014). Multilocus adaptation associated with heat resistance in reef-building corals. *Curr Biol* **24**(24)**:** 2952-2956.

Bierne N, Patrice D, Boudry P, Bonhomme F (2002). Assortative Fertilization and Selection at Larval Stage in the *Mussels Mytilus edulis* and *M . galloprovincialis*. *Evolution* **2**: 292–298.

Bierne N, Welch J, Loire E, Bonhomme F, David P (2011). The coupling hypothesis: Why genome scans may fail to map local adaptation genes. *Molecular Ecology* **20**: 2044–2072.

Both C, Bouwhuis S, Lessells C, Visser ME (2006). Climate change and population declines in a long-distance migratory bird. *Nature* **441**(7089)**:** 81-83.

Bubier J, Schläppi M (2004). Cold induction of EARLI1 a putative Arabidopsis lipid transfer protein, is light and calcium dependent. *Plant, Cell and Environment* **27**: 929–936.

Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P *et al* (2012). Biodiversity loss and its impact on humanity. *Nature* **486**(7401)**:** 59-67.

Corlett RT, Westcott DA (2013). Will plant movements keep up with climate change? *Trends in Ecology & Evolution* **28**(8)**:** 482-488.

Costanza R, d'Arge R, De Groot R, Farber S, Grasso M, Hannon B *et al* (1997). The value of the world's ecosystem services and natural capital. *Nature* **387**(6630)**:** 253-260.

Dattolo E, Marïn-Guirao L, Ruiz JM, Procaccini G (2017). Long-term acclimation to reciprocal light conditions suggests depth-related selection in the marine foundation species Posidonia oceanica. *Ecology and Evolution* **7**: 1148–1164.

D'Esposito D, Orrù L, Dattolo E, Bernardo L, Lamontara A, Orsini L *et al* (2016). Transcriptome characterisation and simple sequence repeat marker discovery in the seagrass *Posidonia oceanica*. *Scientific Data* **3:** 160115.

Diaz-Almela E, Marbà N, Alvarez E, Balestri E, Ruiz-Fernandez JM, Duarte CM (2006). Patterns of seagrass (*Posidonia oceanica*) flowering in the Western Mediterranean. *Mar Biol* **148**(4)**:** 723-742.

Excoffier L, Lischer HE (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**(3)**:** 564-567.

Feng H, Chen Q, Feng J, Zhang J, Yang X, Zuo J (2007). Functional characterization of the *Arabidopsis* eukaryotic translation initiation factor 5A-2 that plays a crucial role in plant growth and development by regulating cell division, cell growth, and cell death. *Plant physiology* **144**(3)**:** 1531-1545.

Foll M, Gaggiotti O (2008). A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics* **180**(2)**:** 977-993.

Franks SJ, Weber JJ, Aitken SN (2014). Evolutionary and plastic responses to climate change in terrestrial plant populations. *Evolutionary Applications* **7**(1)**:** 123-139.

Franssen SU, Gu J, Winters G, Huylmans A-K, Wienpahl I, Sparwel M *et al* (2014). Genome-wide transcriptomic responses of the seagrasses *Zostera marina* and *Nanozostera noltii* under a simulated heatwave confirm functional types. *Marine genomics* **15**(0)**:** 65-73.

Giakoumi S, Halpern BS, Michel LN, Gobert S, Sini M, Boudouresque CF *et al* (2015). Towards a framework for assessment and management of cumulative human impacts on marine food webs. *Conserv Biol* **29.4** 1228-1234.

Hessen DO, Daufresne M, Leinaas HP (2013). Temperature-size relations from the cellular-genomic perspective. *Biological Reviews* **88**(2)**:** 476-489.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E *et al* (2007). Coral reefs under rapid climate change and ocean acidification. *Science* **318**(5857)**:** 1737-1742.

Hopkins MT, Lampi Y, Wang T-W, Liu Z, Thompson JE (2008). Eukaryotic translation initiation factor 5A is involved in pathogen-induced cell death and development of disease symptoms in *Arabidopsis*. *Plant physiology* **148**(1)**:** 479-489.

630 Jahnke M, Alcoverro T, Lavery PS, McMahon KM, Procaccini G (2015a).
631 Should we sync? Seascape-level genetic and ecological factors determine
632 seagrass flowering patterns. *Journal of Ecology* **103**(6)**:** 1464-1474.

633 Jahnke M, Casagrandi R, Melià P, Schiavina M, Schultz S, Zane L *et al*
634 (2017). Potential and realized connectivity of the seagrass *Posidonia*
635 *oceanica* and their implication for conservation. *Diversity and Distributions*
636 **23**: 1423-1424.

637 Jahnke M, Olsen JL, Procaccini G (2015b). A meta-analysis reveals a
638 positive correlation between genetic diversity metrics and environmental
639 status in the long-lived seagrass *Posidonia oceanica*. *Molecular Ecology*
640 **24**(10)**:** 2336-2348.

641 Jombart T (2008). adegenet: a R package for the multivariate analysis of
642 genetic markers. *Bioinformatics* **24**(11)**:** 1403-1405.

643 Jombart T, Devillard S, Balloux F (2010). Discriminant analysis of principal
644 components: a new method for the analysis of genetically structured
645 populations. *BMC Genetics* **11**(1)**:** 94.

646 Jorda G, Marbà N, Duarte CM (2012). Mediterranean seagrass vulnerable to
647 regional climate warming. *Nature Climate Change* **2**(11)**:** 821-824.

648 Jueterbock A, Franssen SU, Bergmann N, Gu J, Coyer JA, Reusch TB *et al*
649 (2016). Phylogeographic differentiation versus transcriptomic adaptation to
650 warm temperatures in *Zostera marina*, a globally important seagrass.
651 *Molecular Ecology* **25**(21)**:** 5396-5411

652 Kosová K, Vítámvás P, Urban MO, Prášil IT, Renaut J (2018). Plant Abiotic
653 Stress Proteomics: The Major Factors Determining Alterations in Cellular
654 Proteome. *Frontiers in Plant Scince* **9**: 1–22.

655 Langmead B, Trapnell C, Pop M, Salzberg SL (2009). Ultrafast and
656 memory-efficient alignment of short DNA sequences to the human genome.
657 *Genome biology* **10**(3)**:** 1.

658 Love MI, Huber W, Anders S (2014). Moderated estimation of fold change
659 and dispersion for RNA-seq data with DESeq2. *Genome biology* **15**(12)**:** 1-
660 21.

661 Marbà N, Duarte CM (1998). Rhizome elongation and seagrass clonal
662 growth. *Marine Ecology Progress Series* **174:** 269-280.

663 Marín-Guirao L, Entrambasaguas L, Dattolo E, Ruiz JM, Procaccini G
664 (2017). Molecular Mechanisms behind the Physiological Resistance to

Intense Transient Warming in an Iconic Marine Plant. *Frontiers in Plant Science* **8**.

Marin-Guirao L, Ruiz JM, Dattolo E, Garcia-Munoz R, Procaccini G (2016). Physiological and molecular evidence of differential short-term heat tolerance in Mediterranean seagrasses. *Scientific Reports* **6:** 28615.

McCarthy JD, Yunshun C, Smyth KG (2012). Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Research* **40:** 4288-4297.

Migliaccio M, De Martino F, Silvestre F, Procaccini G (2005). Meadow-scale genetic structure in *Posidonia oceanica*. *Marine Ecology Progress Series* **304:** 55-65.

Nardelli BB, Tronconi C, Pisano A, Santoleri R (2013). High and Ultra-High resolution processing of satellite Sea Surface Temperature data over Southern European Seas in the framework of MyOcean project. *Remote Sensing of Environment* **129:** 1-16.

Nevarez L, Vasseur V, Le Drean G, Tanguy a., Guisle-Marsollier I, Houlgatte R, *et al.* (2008). Isolation and analysis of differentially expressed genes in *Penicillium glabrum* subjected to thermal stress. *Microbiology* **154**: 3752–3765.

Orsini L, Spanier KI, De Meester L (2012). Genomic signature of natural and anthropogenic stress in wild populations of the waterflea *Daphnia magna*: validation in space, time and experimental evolution. *Molecular Ecology* **21**(9)**:** 2160-2175.

Orsini L, Vanoverbeke J, Swillen I, Mergeay J, Meester L (2013). Drivers of population genetic differentiation in the wild: isolation by dispersal limitation, isolation by adaptation and isolation by colonization. *Molecular Ecology* **22**(24)**:** 5983-5999.

Peakall R, Smouse PE (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* **28**(19)**:** 2537-2539.

Procaccini G, Orsini L, Ruggiero MV, Scardi M (2001). Spatial patterns of genetic diversity in *Posidonia oceanica*, an endemic Mediterranean seagrass. *Molecular Ecology* **10**(6)**:** 1413-1421.

Procaccini G, Ruggiero MV, Orsini L (2002). Genetic structure and distribution of microsatellite population genetic diversity in *Posidonia*

700 *oceanica* in the Mediterranean basin. *Bulletin of Marine Science* **71**(3)**:**
701 1291-1297.

702 Procaccini G, Ruocco M, Marín-Guirao L, Dattolo E, Brunet C, D'Esposito
703 D *et al* (2017). Depth-specific fluctuations of gene expression and protein
704 abundance modulate the photophysiology in the seagrass *Posidonia*
705 *oceanica*. *Scientific Reports* **7**: 42890.

706 Procaccini G, Waycott M (1998). Brief communication. Microsatellite loci
707 identified in the seagrass *Posidonia oceanica* (L.) Delile. *Journal of*
708 *Heredity* **89**(6)**:** 562-568.

709 R Development Core Team (2012). R: A language and environment for
710 statistical computing. *R Foundation for Statistical Computing, Vienna,*
711 *Austria* **ISBN 3-900051-07-0, URL http://www.R-project.org/**.

712 Rivetti I, Fraschetti S, Lionello P, Zambianchi E, Boero F (2014). Global
713 warming and mass mortalities of benthic invertebrates in the Mediterranean
714 Sea. *PloS one* **9**(12)**:** e115655.

715 Ruggiero M, Turk R, Procaccini G (2002). Genetic identity and
716 homozygosity in North-Adriatic populations of *Posidonia oceanica*: An
717 ancient, post-glacial clone? *Conserv Genet* **3**(1)**:** 69-72.

718 Rundle, HD, Nosil, P (2005). Ecological speciation. *Ecology Letters*, **8**,
719 336–352.

720 Sanford E, Kelly MW (2011). Local adaptation in marine invertebrates. *Ann*
721 *Rev Mar Sci* **3:** 509-535.

722 Serra IA, Innocenti AM, Di Maida G, Calvo S, Migliaccio M, Zambianchi E
723 *et al* (2010). Genetic structure in the Mediterranean seagrass *Posidonia*
724 *oceanica*: disentangling past vicariance events from contemporary patterns
725 of gene flow. *Molecular Ecology* **19**(3)**:** 557-568.

726 Shi Y, Zhang X, Xu ZY, Li L, Zhang C, Schläppi M, *et al.* (2011). Influence
727 of EARLI1-like genes on flowering time and lignin synthesis of *Arabidopsis*
728 *thaliana*. *Plant Biology* **13**: 731–739.

729 Short FT, Polidoro B, Livingstone SR, Carpenter KE, Bandeira S, Bujang
730 JS *et al* (2011). Extinction risk assessment of the world's seagrass species.
731 *Biological Conservation* **144**(7)**:** 1961-1971.

732 Schluter, D (2009). Evidence for ecological speciation and its alternative.
733 *Science*, **323**, 737–741.

734 Smith DM, Cusack S, Colman AW, Folland CK, Harris GR, Murphy JM
735 (2007). Improved surface temperature prediction for the coming decade
736 from a global climate model. *Science* **317**(5839)**:** 796-799.

737 Tewksbury JJ, Huey RB, Deutsch CA (2008). Putting the heat on tropical
738 animals. *SCIENCE-NEW YORK THEN WASHINGTON*- **320**(5881)**:** 1296.

739 Tomasello A, Di Maida G, Calvo S, Pirrotta M, Borra M, Procaccini G
740 (2009). Seagrass meadows at the extreme of environmental tolerance: the
741 case of *Posidonia oceanica* in a semi-enclosed coastal lagoon. *Marine*
742 *Ecology* **30**(3)**:** 288-300.

743 Van Der Wal J, Murphy HT, Kutt AS, Perkins GC, Bateman BL, Perry JJ *et*
744 *al* (2013). Focus on poleward shifts in species' distribution underestimates
745 the fingerprint of climate change. *Nature Climate Change* **3**(3)**:** 239-243.

746 Van Loon LC, Rep M, Pieterse C (2006). Significance of inducible defense-
747 related proteins in infected plants. *Annual Review Phytopathology* **44:** 135-
748 162.

749 Wang IJ (2013). Examining the full effects of landscape heterogeneity on
750 spatial genetic variation: a multiple matrix regression approach for
751 quantifying geographic and ecological isolation. *Evolution* **67**(12)**:** 3403-
752 3411.

753 Waters JM, Fraser CI, Hewitt GM (2013). Founder takes all: density-
754 dependent processes structure biodiversity. *Trends in ecology & evolution*
755 **28**(2)**:** 78-85.

756 Weisshaar B, Jenkins GI (1998). Phenylpropanoid biosynthesis and its
757 regulation. *Current opinion in plant biology* **1**(3)**:** 251-257.

758 Xu J, Zhang B, Jiang C, Ming F (2011). RceIF5A, encoding an eukaryotic
759 translation initiation factor 5A in *Rosa chinensis*, can enhance
760 thermotolerance, oxidative and osmotic stress resistance of *Arabidopsis*
761 *thaliana*. *Plant Molecular Biology* 75: 167–178.

762 Zhang Y, Schläppi M (2007). Cold responsive EARLI1 type HyPRPs
763 improve freezing survival of yeast cells and form higher order complexes in
764 plants. *Planta* **227**: 233–243.

765

Table 1 Molecular variance.

Analysis molecular variance (AMOVA) for neutral and outlier loci. The variance is partitioned in the following levels: i) among groups, where groups are either populations along the depth or the latitudinal gradient; ii) within groups, where the groups are either populations at the same depth or at the same latitude and; iii) individuals within populations. Significant values ($P < 0.001$) are in italics (10,000 permutations).

	Hierarchical level	**among groups**	**within groups**	**within pops**
Neutral loci	depth	0%	*22%*	*78%*
	latitude shallow	*6%*	*17%*	*77%*
	latitude deep	*9%*	*19%*	*72%*
Gradient shared loci	depth	0%	0%	100%
	latitude shallow	0%	0%	100%
	latitude deep	0%	0%	100%
Latitude loci	depth	0%	*4%*	*96%*
	latitude shallow	0%	*4%*	*96%*
	latitude deep	0%	*4%*	*96%*
Bathymetric loci	depth	0%	1%	*99%*
	latitude shallow	0%	0%	*100%*
	latitude deep	0%	1%	*99%*

Table 2 Correlation among genetic, environmental and geographic variation.

Multiple matrix regression with randomization analysis (MMRR) showing correlations between geographic distance (measured between sampling locations using the shortest path over the sea without crossing land), genetic distance (measured as F_{ST} at microsatellite loci) and environmental distance (measured as the difference between locations of averaged temperature between 1981 and 2012). Significant P-values are in italics.

		Intercept	Geographic distance	Temperature
F_{ST} **neutral**	**Coefficients**	-0.157	0.116	0.541
	t-statistic	-0.570	0.467	1.809
	p-value	0.817	0.669	*0.003*
F_{ST} **gradient shared loci**	**Coefficients**	-0.422	-0.020	-0.016
	t-statistic	-17.164	-0.891	-0.597
	p-value	0.427	0.387	0.102
F_{ST} **latitude loci**	**Coefficients**	0.484	-0.049	-0.360
	t-statistic	1.105	-0.123	-0.758
	p-value	0.157	0.869	0.305
F_{ST} **bathymetric loci**	**Coefficients**	-0.290	-0.452	0.498
	t-statistic	-0.615	-1.064	0.975
	p-value	0.479	0.280	0.328

Titles and legends to figures

Figure 1. *Posidonia oceanica* sampling.

Posidonia oceanica was sampled from six geographic locations in the Mediterranean Sea along a latitudinal transect of 1,000 Km at two different depths (5 m and 20-25 m).

Figure 2. Population structure analysis.

Discriminant Analysis of Principal Component (DAPC) displaying the total set of 12 *Posidonia oceanica* populations, including shallow and deep stands. The optimal number of principal components found for the analysis was eight. Populations from the same geographic location sampled at different depths are labelled with D (deep) and S (shallow). All populations are uniquely color-coded. N/S identifies the boundary between northern and southern populations.

Figure 3. Gene expression analysis.

Heatmaps of differentially expressed genes (DEseq $p\text{-}adj < 0.01$) between the deep and the shallow stands of the Stareso population, grouped in functional categories. a) bHLH (basic/helix-loop-helix), b) cell wall, c) kinases, d) MYB, e) phosphatases, f) secondary metabolism, g) WRKY. S1 and S2 - shallow stand sampled at 12 noon and 6.30pm, respectively; D1

807 and D2 - deep stand sampled at 12 noon and 6.30pm, respectively. Raw
808 zeta-scores were calculated from the DESeq2 normalized counts.
809